REMARKS

Reconsideration of the rejections set forth in the Office action mailed April 7, 2004 is respectfully requested. Claims 1, 4-6 and 13 are currently under examination. No amendments are made with this response.

I. Rejections under 35 U.S.C. §103

Claims 1, 3-6, 13 and 14 were rejected under 35 U.S.C. §103 as being unpatentable over Zyskind *et al.* (U.S. Patent No. 6, 228,579) in view of McKay *et al.* (U.S. Patent No. 6,133,246), Cook (U.S. Patent No. 6,239,265), and Arnold, Jr. *et al.* (U.S. Patent No. 6,060,456). The rejections are respectfully traversed in light of the following remarks.

A. The Invention

The applicant's invention, as embodied in independent claim 1, is directed to a substantially uncharged antisense oligomer containing from 10 to 40 morpholino subunits, each of said subunits supporting a base-pairing moiety effective to bind by Watson-Crick base pairing to a respective nucleotide base, said base-pairing moieties including a targeting nucleic acid sequence at least 10 nucleotides in length which is effective to specifically hybridize to a target sequence which spans the translational start codon for secA protein within the *E. coli* nucleic acid sequence presented as SEQ ID NO: 2,

wherein adjacent subunits are joined by uncharged linkages selected from the group consisting of: uncharged phosphoramidate and phosphorodiamidate, or by charged linkages selected from the group consisting of charged phosphoramidate and phosphorodiamidate, and the ratio of uncharged linkages to charged linkages in the oligomer is at least 4:1.

Results discussed at page 28, line 21 to page 29, line 6 of the specification show that morpholino oligomers directed to various bacterial proteins inhibited growth of *E. coli* and *E. faecium* by up to 80%, at a concentration of 1.0 μM. The oligomers were effective when delivered in a conventional buffer vehicle (as described on page 34), suggesting that they are actively transported into the cells. As shown in Fig. 4B of the specification, an antisense morpholino oligomer of the claim, having the sequence presented as SEQ ID NO: 47, inhibited growth of *E. coli* by about 60% at a concentration of 0.1 μM, and by about 70% at a

concentration of 1.0 μM.

B. Initial Remarks

Applicants note the following:

(1) Contrary to the description of the claims in the Office Action (page 3), the linkages recited in claim 1 do not include "carbonate, carbamate, amide", "phosphate or phosphorothioate" linkages.

(2) The current rejection under §103(a) is very similar to that made in the Office Action dated October 25, 2002. The primary difference is that the reference McKay *et al.* has been added. The applicant feels that this reference adds very little with respect to the pending claims, as discussed further below.

In response to the earlier §103(a) rejection, the broad claim was narrowed by removing the above-noted linkage types (response filed April 24, 2003). In the subsequent Office Action, dated September 10, 2003, the claims were found free of the prior art.

In the response to the §112 rejection in the September 2003 Office Action, the claims were again amended, but nothing in these amendments broadened the scope of the claims. (For example, the phrase "a target sequence containing a translational start codon within a bacterial nucleic acid sequence which encodes an *E. coli secA* protein" was replaced with "a target sequence which spans the translational start codon for *secA* protein within the *E. coli* nucleic acid sequence presented as SEQ ID NO: 2"). It is therefore surprising to the applicant to find the claims again rejected under §103(a), over essentially the same set of cited references which were previously overcome.

C. The Cited Art

Zyskind *et al.* is directed to the use of exogenous nucleic acids which "produce antisense inhibitors of endogenous complementary mRNAs in a microorganism", for "identifying endogenous microbial proliferation genes" of the microorganism (Abstract). Accordingly, the "exogenous nucleic acid" is typically a DNA sequence effective to express a sense or antisense molecule (see e.g. column 2, lines 25-27; column 5, lines 61-67; column 6, lines 59-61; and Examples 1-3). All three Examples employ plasmid DNA hundreds of basepairs in length.

The patent specification also devotes approximately one column to a discussion of antisense oligonucleotides. The reference teaches that such antisense oligonucleotides, typically 10-50 nucleotides in length, may be "of the type found in nature" (column 8, lines 37, 60-63), i.e. phosphodiesters, or they may be analogs such as phosphorothioates, methylphosphonates, or peptide nucleic acids (column 8, line 63 to column 9, line 40). There is no mention of morpholino oligomers.

Nor is there any guidance which would provide a reasonable expectation that such oligonucleotides would enter bacterial cells. As noted above, the working examples are directed to the use of plasmid DNA, typically 500-1000 bp in length, which is transfected and expressed in the cells (see Example 1).

Example 3 describes expression of sense secA RNA in bacteria. The proposed mechanism of inhibition is described at column 18, lines 9-25: "the sense RNA produced by pJB3 after induction could be binding and potentially sequestering most of the secA protein..." (lines 18-20). This is clearly not an antisense mechanism.

The reference goes on to state that an "antisense RNA complementary to secA mRNA would also be expected to inhibit bacterial proliferation. The nucleotide sequence of such an antisense RNA is shown in FIG. 12." Figure 12 shows an 836-nucleotide RNA sequence. There is no evidence that this sequence did in fact inhibit proliferation. Even if it did, this would not provide a reasonable expectation that the 10- to 40-subunit morpholino oligomers now claimed would penetrate bacterial cells and inhibit bacterial proliferation, as shown in the applicant's disclosure.

McKay *et al.* is directed to treatment of diseases or disorders in animals by antisense inhibition of Jun N-terminal kinase (see Abstract).

The patent contains an extensive general discussion of antisense oligonucleotides, including typical target regions and dozens of possible structural modifications (columns 7-11), including a brief mention of morpholino backbone structures. However, the oligonucleotides actually employed in the Examples are phosphorothioates or phosphorothioate-phosphodiester chimeras ("gapmers" or "wingmers"). (Example 1 refers to synthesis of PNA's, but these do not appear in the remaining Examples.)

There is nothing in the reference pertaining to bacterial proteins or the use of antisense to inhibit bacterial growth. Nor is there any clear guidance towards the use of substantially uncharged morpholino antisense oligomers.

Arnold, Jr. *et al.* describes chimeric antisense oligonucleotides which are RNaseH-activating (e.g. Abstract; Field of the Invention; Summary of the Invention, column 4, lines 53-54). The exemplified compounds generally have a central "RNaseH Activating Region" having charged linkages selected from phosphorothioate, phosphodiester, and phosphorodithioate (see Table, column 7 of reference; also Tables at columns 45-48, 53-60, etc.).

As noted in the applicant's specification at page 13, lines 24-28, morpholino oligomers are considered RNaseH inactive; therefore, this reference teaches away from the use of the claimed oligomers for antisense applications.

Cook is directed to the preparation of oligonucleotides having chiral phosphorus-containing linkages, such as phosphorothioate, methylphosphonate, phosphotriester, or phosphoramidate, in a stereoselective manner, to produce compounds of "relatively high enantiomeric purity". See, for example, column 9, lines 22-29. As shown at column 8 of the patent, the oligonucleotides are composed of ribose or deoxyribose subunits. There is no description of morpholino oligomers as claimed.

C. Analysis

The cited references, taken alone or in combination, would not motivate one skilled in the art to employ substantially uncharged morpholino oligomers targeted to bacterial proteins, as presently claimed. The emphasis of the teachings of these references with respect to antisense technology is, on the contrary, towards the use of substantially charged, RNAseH-activating oligonucleotides such as phosphodiesters, phosphorothioates, modified phosphorothioates, or chimeras of these types. Moreover, only Zyskind includes any teaching with respect to bacterial inhibition, and shows such inhibition only via transfection with plasmid DNA.

The cited references, even if combined, provide no suggestion to employ a substantially uncharged morpholino oligomer targeting a start codon region of *E. coli* secA protein; nor do

they provide any expectation of success.

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

II. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4403.

Respectfully submitted,

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